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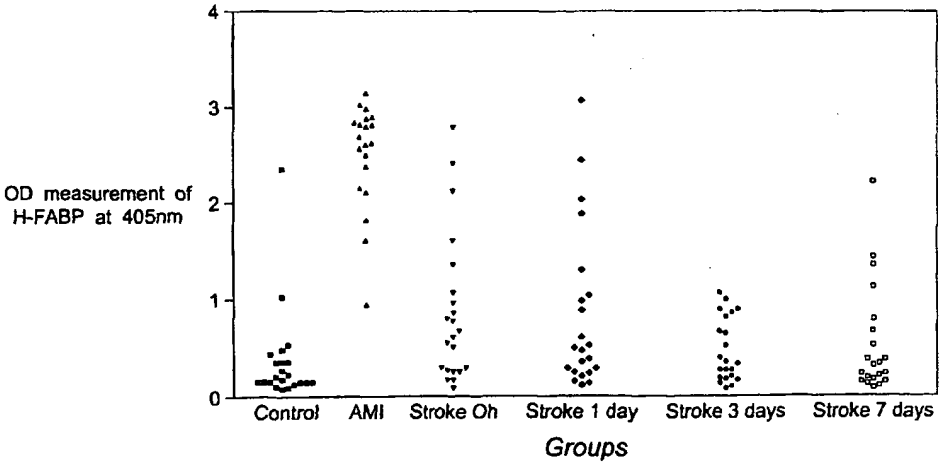
(54) Title: DIAGNOSTIC ASSAY FOR STROKE



(57) Abstract: Heart and brain fatty acid binding proteins (H-FABP, B-FABP) are markers for stroke. The invention provides a diagnostic assay for either of these markers, preferably by ELISA using a anti-H-FABP or B-FABP antibody. Since H-FABP is also a marker for acute myocardial infarction (AMI), to distinguish stroke from AMI requires an assay specific to AMI, e.g. using troponin-1 or CK-MB as a marker, also to be carried out.

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"DIAGNOSTIC ASSAY FOR STROKE"

BACKGROUND OF THE INVENTION

Field of the invention

This invention is in the field of diagnostic assay using a protein or an antibody thereto.

Description of the related art

Stroke has the third highest death-rate in industrial countries. It results from either a permanent or a transient reduction in cerebral blood flow. This reduction in flow is, in most cases, caused by the arterial occlusion due to either an embolus or a local thrombosis. Depending on the localisation of brain injury and the intensity of necrosed neurones, stroke symptoms can become a life handicap for patients and the death rate from stroke events approaches 30%.

Recently, S100B was described as a potential biochemical marker for stroke diagnosis, see U.Missler *et al.*, "S100 protein and neuron-specific enolase concentrations in blood as indicators of infarct volume and prognosis in acute ischemia stroke", Stroke 1997;28:1956-60. However, S100B has also been reported as a useful marker for early detection of metastases of melanoma and cerebral complications from head injury and cardiac surgery. Thus, the sensitivity and specificity of the S100B test were limited to 44% and 67%, respectively, see M.Takahashi *et al.*, "Rapid and sensitive immunoassay for the measurement of serum S100B using isoform-specific monoclonal antibody", Clin. Chem. 1999;45:1307-11. Development of new stroke markers would help clinicians to establish early diagnosis and thus to avoid a potential relapse of the patient.

SUMMARY OF THE INVENTION

It has now surprisingly been found that two fatty acid binding proteins (FABP), known as heart (H-FABP) and brain (B-FABP), are markers for stroke. Thus, the

invention provides a method of diagnostic assay for stroke or the possibility thereof in a sample of body fluid taken from a patient suspected of suffering from a stroke, which comprises determining the concentration of heart or brain fatty acid binding protein (H-FABP or B-FABP) in the sample. The concentration thus determined is used to make or assist in making a diagnosis.

Conveniently the method is carried out using an antibody to H-FABP or B-FABP, whereby the extent of the reaction between the antibody and the FABP in the sample is assayed and related to the concentration of FABP in the sample.

The present invention enables an assay of high sensitivity, specificity and predictive positive value for stroke to be carried out. "Sensitivity" is defined as the percentage of true positives given by the assay on samples taken from patients in whom clinical examination has confirmed stroke. It is reckoned as % True positives/ (True positives + False negatives). "Specificity" means the percentage of true negatives given by the assay on control samples, i.e. from patients in whom clinical examination has not revealed stroke. It is reckoned as % True negatives/(False positives + True negatives). "Predictive positive value" means the ratio % True positives/ (True positives + False positives).

H-FABP is a known marker of acute myocardial infarction (AMI), see J.Ishii *et al.*, "Serum concentrations of myoglobin vs human heart-type cytoplasmic fatty-acid binding protein in early detection of acute myocardial infarction", Clinical Chemistry 1997;43 1372-1378. Therefore, in order to use an assay for H-FABP for stroke to better advantage, it is desirable to perform another kind of assay for AMI (one in which the marker is not a FABP) in order to eliminate

from the diagnosis for stroke those patients who are positive in the AMI assay.

Thus, in a particular embodiment, the invention provides a method which comprises determining the concentration of H-FABP in a first assay, as defined above, whereby a positive result indicates the possibility of either a stroke or acute myocardial infarction, and which further comprises carrying out a second diagnostic assay, for acute myocardial infarction (AMI) only, whereby a positive result in the H-FABP assay and a negative result in the assay for AMI indicates that the patient might be suffering from a stroke. Assays using Troponin-I and Creatine Kinase-MB (CK-MB) as early biochemical markers of acute myocardial infarction (AMI) are well known and suitable for the above purpose. They can be carried out in plasma, serum or blood. Of course, the terms "first" and "second" are merely convenient labels: the two assays can be carried out in either order.

A similar H-FABP and also a brain-specific fatty acid binding protein (B-FABP) have been found in the brain of mice, see L. Pu *et al.*, Molecular and Cellular Biochemistry 1999;198 69-78. Brain H-FABP (not to be confused with B-FABP) is believed to differ from heart H-FABP by a single amino acid substitution. However, B-FABP differs considerably. P.A.Sellner *et al.*, " Development role of fatty acid binding proteins in mouse brain" Dev. Brain Res. 1995;89:33-46 estimated the DNA homology at 69%, while A.Schreiber *et al.*, "Recombinant human heart-type fatty acid binding protein as standard in immunochemical assays" mention 64% amino acid sequence homology and that a monoclonal antibody to human H-FABP is cross-reactive with human B-FABP to the extent of only 1.7%.

Now that the present inventors have found that H-FABP is a marker for stroke, it is a very reasonable prediction that B-FABP will also be. Since B-FABP is specific to brain tissue and does not appear to react significantly with a monoclonal antibody to H-FABP, it will not give positives for AMI, making a separate assay for AMI unnecessary.

BRIEF DESCRIPTION OF THE DRAWING

The sole Figure is a graphic representation on the y-axis of H-FABP concentration represented by optical density measurement at 405 nm, as determined by the method of the invention, for (a) the control group having neither stroke nor AMI (b) the group having AMI and (c) the stroke group, at four time points after admission (0 hours, 1 day, 3 days and 7 days).

DESCRIPTION OF PREFERRED EMBODIMENTS

For the method of assay, the sample can be taken from the blood, plasma or serum of the patient. The marker, H-FABP or B-FABP, is preferably measured by an immunoassay, using a specific antibody to H-FABP and measuring the extent of the antigen (H-FABP or B-FABP)/antibody interaction. For the diagnosis of human patients, the antibody is preferably anti-human H-FABP or B-FABP. Similarly, if the patient is an animal the antibody should be to the H-FABP or B-FABP of the same animal variety, e.g. anti-equine H-FABP or B-FABP if the patient is a horse. It may be a monoclonal antibody or an engineered antibody. Conveniently a mouse anti-human, anti-equine etc. monoclonal antibody is used. Antibodies to H-FABP are known, e.g. 66E2 and 67D3 described by W. Roos *et al.*, "Monoclonal antibodies to human heart type fatty acid-binding protein", J. Immunol. Methods 1995;183 149-153, are commercially available. Also, the usual Köhler-Milstein method may be used to raise H-FABP or B-FABP antibodies. The source of protein for this purpose

can be the naturally derived or recombinant DNA-prepared protein. Recombinant human H-FABP and B-FABP have been described by A.Schreiber *supra* and F.Shimizu *et al.*, "Isolation and expression of a cDNA for human brain fatty acid binding protein (B-FABP)", Biochim. Biophys. Acta 1997;1354:24-28, respectively. Less preferably, the antibody may be polyclonal.

Any known method of immunoassay may be used. A sandwich assay is preferred. In this method, an antibody (e.g. polyclonal) to the FABP is bound to the solid phase such as a well of a plastics microtitre plate, and incubated with the sample and with a labelled second antibody specific to the H-FABP or B-FABP to be detected. Alternatively, an antibody capture assay (also called "indirect immunoassay") could be used. Here, the test sample is allowed to bind to a solid phase, and the anti-FABP antibody (polyclonal or monoclonal) is then added and allowed to bind. If a polyclonal antibody is used in this context, it should desirably be one which exhibits a low cross-reactivity with other forms of FABP. After washing away unbound material, the amount of antibody bound to the solid phase is determined using a labelled second antibody, anti- to the first.

A direct assay could be performed by using a labelled anti-FABP antibody. The test sample is allowed to bind to the solid phase and the anti-FABP antibody is added. After washing away unbound material, the amount of antibody bound to the solid phase is determined. The antibody can be labelled directly rather than *via* a second antibody.

In another embodiment, a competition assay could be performed between the sample and a labelled FABP or a peptide derived therefrom, these two antigens being in competition for a limited amount of anti-FABP antibody bound to a solid support. The labelled FABP or peptide

could be pre-incubated with the antibody on the solid phase, whereby the FABP in the sample displaces part of the FABP or peptide thereof bound to the antibody.

In yet another embodiment, the two antigens are allowed to compete in a single co-incubation with the antibody. After removal of unbound antigen from the support by washing, the amount of label attached to the support is determined and the amount of protein in the sample is measured by reference to standard titration curves established previously.

Throughout, the label is preferably an enzyme. The substrate for the enzyme may be colour-forming, fluorescent or chemiluminescent. Alternatively, the label may be a radioisotope or fluorescent, e.g. using conjugated fluorescein.

The enzyme is preferably alkaline phosphatase or horseradish peroxidase and can conveniently be used colorimetrically, e.g. using p-nitrophenyl phosphate as a yellow-forming substrate with alkaline phosphatase.

For a chemiluminescent assay, the antibody can be labelled with an acridinium ester or horseradish peroxidase. The latter is used in enhanced chemiluminescent (ECL) assay. Here, the antibody, labelled with horseradish peroxidase, participates in a chemiluminescent reaction with luminol, a peroxide substrate and a compound which enhances the intensity and duration of the emitted light, typically 4-iodophenol or 4-hydroxycinnamic acid.

An amplified immunoassay such as immuno-PCR can be used. In this technique, the antibody is covalently linked to a molecule of arbitrary DNA comprising PCR primers, whereby the DNA with the antibody attached to it is amplified by the polymerase chain reaction. See E. R. Hendrickson *et al.*, Nucleic Acids Research 1995; 23, 522-529 (1995) or T. Sano et al., in "Molecular Biology

and Biotechnology" ed. Robert A. Meyers, VCH Publishers, Inc. (1995), pages 458 - 460. The signal is read out as before.

In a particularly preferred procedure, an enzyme-linked immunosorbent assay (ELISA) was developed to detect H-FABP. Since H-FABP is a marker for AMI as well, Troponin-I or CK-MB concentrations were assayed in order to exclude any heart damage. As described in the Example, These assays were assessed in serial plasma samples, from 22 patients lacking AMI and stroke, 20 patients with AMI and 22 patients with confirmed stroke at four times points after the admission at the medical centre. The sensitivity, specificity and predictive positive value for H-FABP in stroke were 59.1%, 90.9% and 86.7% respectively. Only one out of 22 stroke patients had increased H-FABP and Troponin-I expression. Thus, H-FABP detection combined with the Troponin-I or CK-MB assay provide a useful marker of stroke diagnosis or brain damage.

The use of a rapid microparticle-enhanced turbidimetric immunoassays, developed for H-FABP in the case of AMI, M.Robers *et al.*, "Development of a rapid microparticle-enhanced turbidimetric immunoassay for plasma fatty acid-binding protein, an early marker of acute myocardial infarction", Clin. Chem. 1998;44:1564-1567, should drastically decrease the time of the assay. Thus, the full automation in a widely used clinical chemistry analyser such as the COBAS™ MIRA Plus system from Hoffmann-La Roche, described by M.Robers *et al. supra*, or the AxSYM™ system from Abbott Laboratories, should be possible and applied for routine clinical diagnosis of stroke.

The H-FABP or B-FABP concentrations can be measured by other means than immunoassay. For example, the sample

can be subjected to 2D-gel electrophoresis and the amount of the FABP estimated by densitometric scanning of the gel or of a blot therefrom. However, it is desirable to carry out the assay in a rapid manner, so that the patient can be treated promptly.

The following Example illustrates the invention.

EXAMPLE

Materials And Methods

Patients

The study population consisted of 22 age-and-gender matched control patients (Control group), 20 confirmed AMI patients (AMI group) and 22 confirmed stroke patients (Stroke group). The Control group included 14 men, mean age 66, range 34-86 years, and 8 women, mean age 63, range 51-81 years. The AMI group included 16 men, mean age 65, range 29-90 years, and 4 women, mean age 72, range 66-81 years. The Stroke group included 14 men, mean age 65, range 30-87 years, and 8 women, mean age 64, range 51-85 years. Four blood samples were collected for each patient of the Stroke group after admission (t_0=0h; t_1=1 day, t_3=3 days, t_7=7 days). Blood samples were collected in dry heparin-containing tubes. After centrifugation at 1500g for 15min at 4°C, the plasma samples were stored as aliquots at -20°C until analysis. Patients from the Stroke group underwent serial clinical evaluations by neurologists in order to confirm stroke diagnosis. Patients from AMI group were admitted to the hospital with a confirmed AMI (Troponin-I concentration >2ng/ml). A clinical evaluation was performed on all the patients from the control group to exclude Stroke and AMI.

Measurement of brain and heart H-FABP

H-FABP concentrations were measured in plasma by a sandwich ELISA. A 96-well polystyrene microplate (NUNC) was coated with 100µl/well polyclonal goat anti human

muscle-FABP (Spectral Diagnosis HC, Ontario, USA), 20.4ng/ml in carbonate buffer 0.1M pH 9.6, overnight at 4°C. The plate was automatically washed with PBS (15mM Na_2PO_4-120mM NaCl-2.7mM KCl pH 7.4, Sigma) on a BioRad NOVAPATH™ washer. Every washing step was performed with fresh PBS. Non-specific binding sites were blocked with 200µl/well 2% casein in carbonate buffer for 2h at 37°C. After the washing step, the samples were pipetted in duplicate at 100µl/well. The plate was incubated 2h at 37°C. After the washing step, 100µl/well of mouse anti-human Heart FABP (clone 66E2, HyCult Biotechnology BV, Uden, Netherlands), 0.3ng/ml in PBS-1%BSA, were incubated for 1h at room temperature (R.T.) with shaking. After the washing step, 100µl/well of phosphatase-labelled anti-mouse immunoglobulin (Dako, Denmark), 15ng/ml in PBS, were incubated 1h 30min at R.T. with shaking. After the washing step, 50µl/well of phosphatase substrate, 1.5mg/ml para-nitrophenylphosphate in diethanolamine, were incubated 30min. The reaction was stopped with 100µl/well 1M NaOH. Colour development was measured with a microplate reader at a wavelength of 405nm.

CK-MB and Troponin-I measurement

AMI was diagnosed by clinical evaluation and Troponin-I and CK-MB measurements. Samples were centrifuged at 1500g for 15min, and stored at -20°C. Serum CK-MB and Troponin-I concentrations were determined using a fluorescent microparticle enzyme immunoassay (MEIA) with an automated chemical analyser AxSYM™ system (Abbott Laboratories, Abbott Park, IL, USA). The rate of formation of fluorescent products was directly proportional to the amount of Troponin-I in the sample. The detection limit for Troponin-I was 0.3µg/l. CK-MB measurement is proportional to the amount of fluorescent probes and the detection limit was 0.7µg/l.

Statistical analysis

H-FABP concentrations were expressed in optical densitometry (OD) values either as mean ± SD or as median and inter-quartile range. Troponin-I and CK-MB concentrations were expressed in concentration units (ng/ml). The non-parametric Mann-Whitney *U*-test was used to compare in plasma H-FABP, Troponin-I and CK-MB concentrations between groups. PRISM™ software was used to elaborate box/whisker and scatter plots. The 95% confidence intervals (CI) and Receiver Operating Characteristic (ROC) curves, defined by Analyse-it™ software for Microsoft EXCEL™, were used to assess the discriminatory time point of the indicators. See J.M.Murphy *et al.*, "Performance of screening and diagnostic tests", Arch. Gen. Psychiatry 1987;44:550-555.

A univariate Z-test was used to compare the areas under the ROC curves of H-FABP. Differences in sensitivity, specificity and predictive positive value of H-FABP concentrations at each time points were evaluated. $P<0.05$ was considered statistically significant.

Results

Clinical characteristics

Patients from the Stroke group were given a complete clinical evaluation. Ischaemia and haemorrhage were diagnosed with the help of computer tomographic (CT) scan and cerebral IRM response as well as their localisation (data not shown). Stroke diagnosis was confirmed for each patient from the Stroke group. Injury type and localisation did not correlate with H-FABP concentration (data not shown).

Patients from the Control group were admitted to hospital and stroke and AMI were excluded by clinical evaluation.

Patients from the AMI group were admitted to the hospital with confirmed AMI with high Troponin-I levels (>2ng/ml).

Assay results are shown in Table 1 below.

TABLE 1

Assay type	Control Group	AMI Group	Stroke Group			
			0h	1 day	3 days	7 days
H-FABP median (25-75%) OD, 405 nm Significance	0.19 (0.14-0.35)	2.65 (2.27-2.86) ***	0.64 (0.28-1.01) ***	0.46 (0.25-0.98) **	0.37 (0.20-0.76) ns	0.33 (0.18-0.73) ns
Troponin-1 median (25-75%) IU ng/ml Significance	0 (0.0-0.0)	50 (50-359) **	0 (0.0-0.3) ns	0 (0.0-0.2) ns		
CK-MB median (25-75%) IU ng/ml Significance	1 (0.7-0.12)	63 (27-87.5) **	2.7 (1.35-4.05) ns	1.6 (1.3-3.3) ns		

Significance: *** $p<0.001$
** $p<0.01$
ns non-significant

H-FABP plasma concentrations (OD measurement) in the AMI group were significantly higher than in the Control group (Table 1). The AMI group had a H-FABP median

concentration (range 25-75%) of 2.65 (2.27-2.86) while the Control group had a concentration of 0.19 (0.14-0.35). The H-FABP concentration decreases with time after the brain acute event increased. H-FABP median concentration (range 25-75%) in the Stroke group was 0.64 for t_0 (0.28-1.01), 0.46 for t_1 (0.25-0.98), 0.37 for t_3 (0.20-0.76) and 0.33 for t_7 (0.18-0.73). Some overlap exists between the inter-quartile range of the Stroke and the Control groups due to presence of false negatives. The H-FABP concentration distribution was visualised by the scatter plot of the drawing. Receiver Operating Curve plots were made of Sensitivity against Specificity at different times, namely on admission of the patient and at 1, 3 and 7 days after admission. The ROC curves were used to optimise Sensitivity and Specificity and to maximise the sum of Sensitivity and Specificity by choosing and adequate cut-off value in optical density units, representing H-FABP concentration. The plots showed that the best Sensitivity and Specificity for this group of patients was obtained on admission of the patient, with a cut-off value at OD 0.53. Under these conditions, the Sensitivity, Specificity and Predictive Positive Value of H-FABP concentrations were 59.1%, 90.9% and 86.7% respectively. Comparison between ROC curves on admission with those made at the later times did not show any enhancement of sensitivity and specificity values beyond those obtained on admission.

To confirm differences in H-FABP concentrations between AMI and Control groups, CK-MB and Troponin-I were assayed. In addition, in order to discriminate AMI and Stroke, they were also assayed on stroke samples. The Troponin-I and CK-MB concentrations were measured in each group. Troponin-I and CK-MB concentrations in the AMI group were significantly ($P>0.01$) higher than in the Control group. No significant differences of

concentration of these indicators were found between the Control and the Stroke group. ABBOTT laboratories showed that the expected values using the AxSYM™ Troponin-I assay and the AxSYM™ CK-MB assay for AMI diagnosis are determined at the cut off 2ng/ml and 9.3ng/ml respectively. The CK-MB value expected for the control group is up to 3.8ng/ml. At the Troponin-I concentration >2ng/ml, the sensitivity and specificity of the MEIA Troponin-I assay were 93.3% and 94.4%, respectively, at t_1. The median Troponin-I and CK-MB concentrations (25-75%) in the plasma were calculated and are shown in Table 1.

Table 2 summarises the evaluation of the assay.

TABLE 2

Group	Control	AMI	Stroke
No. samples	22	20	22
H-FABP (OD)			
More than 0.531	2	20	13
0.531 or less	20	0	9
Troponin-1 & CK-MB			
AMI diagnosis		20	1
Myocardial suffering without AMI			6
Clinical anamnesis			
Stroke diagnosis			22
Epilepsy	1		
Fracture	2		

In the Control group, two false positives were detected. H-FABP was increased at t_0 (Table 2). One of

these had Troponin-I and CK-MB concentrations at the border between healthy and myocardial pain (3.8ng/ml), indicating that this person should suffer from myocardial muscle lacking AMI. Indeed, one of these had epilepsy and both suffered from several fractures. In the Stroke group, 13 true positives were detected (10 had ischemia and 3 had haemmorage). High H-FABP concentrations were measured and stroke diagnosis was confirmed. Healthy Troponin-I and CK-MB concentrations were measured and allowed the exclusion of an AMI diagnosis, except for one patient. For this exception, clinical evaluation did not detect myocardial suffering and did not correlate with Troponin-I concentration for AMI. H-FABP measured did not allow the discrimination between brain or myocardial pain. In the stroke group, 9 false negatives were detected with low H-FABP levels (6 had ischemia and 3 haemorrage). No explanation was found for these cases. No correlation was found between low H-FABP concentration and clinical evaluation.

Discussion

The above results indicate that H-FABP is a potential marker for stroke diagnosis. Since H-FABP was presented as a marker of acute myocardial infarction few years ago, Stroke and AMI had to be discriminated by another AMI biochemical marker such as Troponin-I or CK-MB. After the discrimination of AMI for stroke patient, the serum H-FABP concentration could be used as a specific marker of stroke.

At the admission, H-FABP assay allowed a sensitivity, a specificity and a predictive positive value (OD response > 0.531) of 59.1%, 90.9% and 86.7% respectively. These values were significantly higher than those of S100B protein for detection of stroke. The sensitivity and the specificity of S100B assay for stroke were of 44% and 67% respectively. The advantage of S100B

analysis was the development of a rapid immunoassay less than 3h. However, the specificity of S100B was not limited to stroke but also metastases of melanoma, cerebral complications from head injury and cardiac surgery. The kinetics of H-FABP in the blood stream were studied by measuring H-FABP at four time points after admission at the screening clinic (t_0 (0h), t_1 (1 day), t (3 days) and t_7 (7 days)) for each patient with confirmed stroke. The maximum H-FABP concentrations were mostly observed at t_0. Variation time between brain onset and admission did not interfere with the result, because the H-FABP concentration remained elevated at t_1. The ROC curve area confirmed the higher H-FABP concentrations at t_0 and t_1 compared to t_3 and t_7. The best characteristics of the assay (sensitivity, specificity) were obtained at t_0.

Acute myocardial infarction is diagnosed with the help of a biochemical marker assay such as for cardiac Troponin-I, Creatine-Kinase MB, myoglobin and, recently, H-FABP assay. Since H-FABP concentrations could indicate AMI, discrimination between AMI and stroke was made with the use of another AMI marker.

Troponin-I is an early marker of AMI or ischemia damage, with the advantage of remaining elevated for several days following AMI. Since the concentration of Troponin-I released reaches a maximum 12-24h after admission, the Stroke group was analysed at t_0 and t_1. The Troponin-I concentration in the AMI group was significantly higher than the cut off value of 2ng/ml. Most of the Stroke group patients showed normal Troponin-I concentration under the cut-off value, which excluded AMI patients of the study, except for one patient. His clinical evaluation did not diagnose any myocardial suffering and this did not correlate with his Troponin-I concentration. In this one case, H-FABP measurement did

not allow discrimination between brain or myocardial pain.

In parallel, the concentration of Creatine Kinase MB in plasma was measured for each patient. This marker is less specific than Troponin-I because it detects any muscle suffering types. Starting at between 2 and 6 hours after the onset of symptoms, CK-MB is released into the blood stream and its concentration therein rises until up to 18 hours after the onset of symptoms. It remains at elevated concentration until about 2 days after the onset of symptoms, after which it falls to normal.

In the Control group, two false positives were detected. One of these had epilepsy which interfere with stroke. Both fell on the floor and broke their femur and foot. Increased CK-MB correlated well with increased H-FABP. Epilepsy could explain the raised H-FABP level. H-FABP allowed a 98.6% correlation with CK-MB assay. Since Troponin-I did not allow the detection of these false positives, Troponin-I and H-FABP measurement gave a 95.3% correlation.

* * * * *

Each of the above cited publications is herein incorporated by reference to the extent to which it is relied on herein.

CLAIMS

1. A method of diagnostic assay for stroke or the possibility thereof in a sample of body fluid taken from a patient suspected of suffering from a stroke, which comprises determining the concentration of heart or brain fatty acid binding protein (H-FABP or B-FABP) in the sample.

2. A method according to Claim 1, wherein the concentration of H-FABP is determined in a first assay, whereby a positive result indicates either a stroke or acute myocardial infarction, and which further comprises carrying out a second diagnostic assay, for acute myocardial infarction (AMI) only, whereby a positive result in the H-FABP assay and a negative result in the assay for AMI indicates that the patient might be suffering from a stroke.

3. A method according to Claim 2, wherein the assay for AMI comprises determining the concentration of troponin-1 or creatine kinase MB in plasma, blood or serum.

4. A method according to Claim 1, 2 or 3, wherein an antibody to H-FABP is used in the assay for H-FABP.

5. A method according to Claim 4, wherein a polyclonal anti-human FABP antibody is used.

6. A method according to any Claim 4 or 5, wherein the assay for H-FABP comprises a sandwich ELISA.

7. A method according to Claim 1, wherein B-FABP or an antibody thereto is used without any assay for AMI in combination therewith.

8. A method according to any preceding Claim, wherein the H-FABP or B-FABP assay is carried out on a blood, serum or plasma sample.

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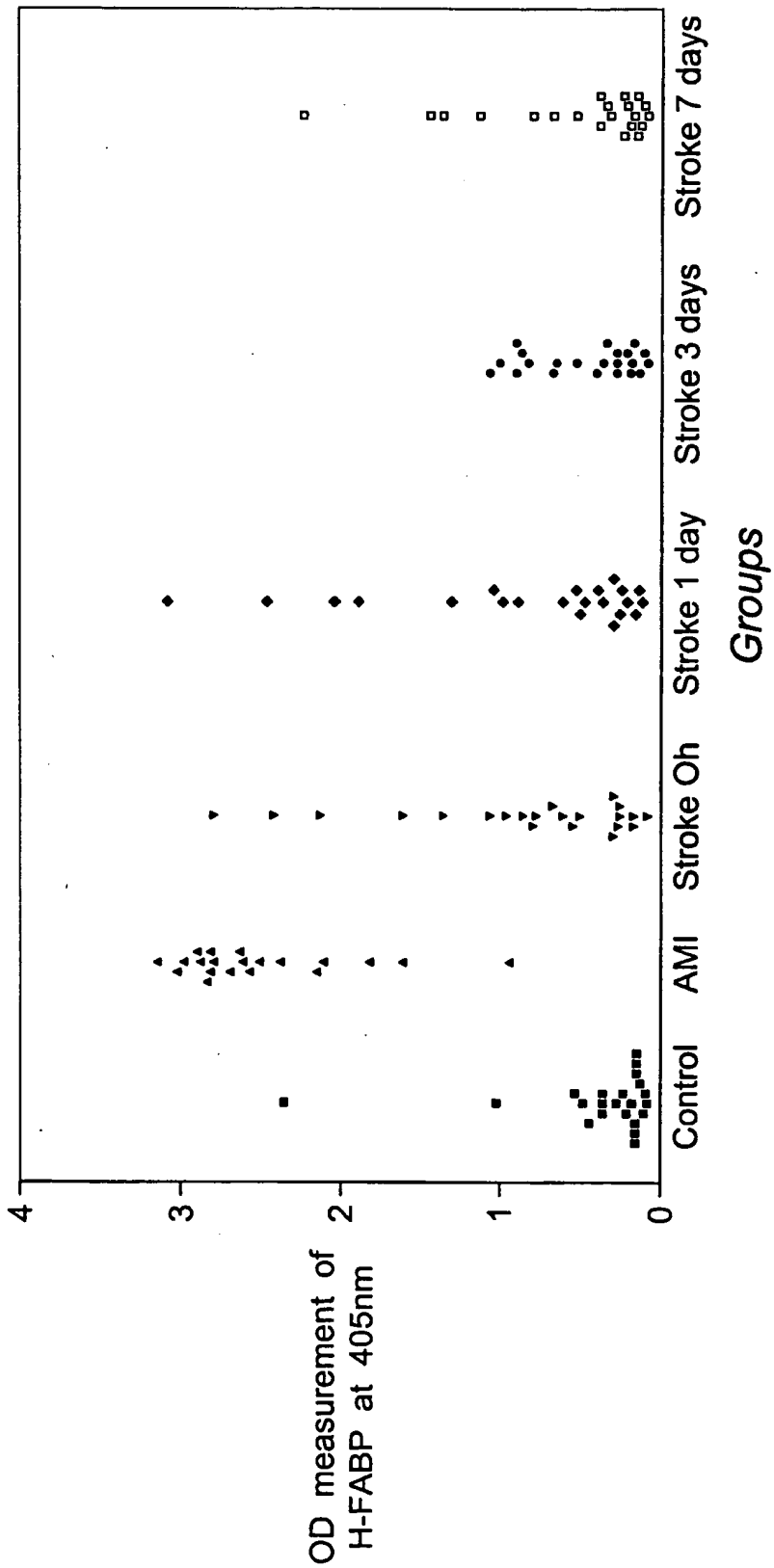
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0
1
2
3
4
Control
AMI
Stroke Oh
Stroke 1 day
Stroke 3 days
Stroke 7 days
Groups